INHIBITION OF SECONDARY CAROTENOID BIOSYNTHESIS DURING DEGREENING OF *CHLORELLA FUSCA* (CHLOROCOCCALES, CHLOROPHYTA) AND IMPLICATIONS FOR GROWTH AND SURVIVAL

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ABSTRACT - Degreening of *Chlorella fusca* under conditions of nitrogen starvation involved many biochemical changes such as loss of photosynthetic pigments and oxygen evolution, as well as synthesis of secondary carotenoids. Addition of the phenylpyridazinone herbicide BASF 44521 [4-chloro-5-methoxy-2-(α, α, α-trifluoro-m-tolyl)3(2H)-pyridazinone] to the degreening medium inhibited the biosynthesis of secondary carotenoids resulting in completely bleached cells that failed to undergo subsequent regreening. Analysis of survivorship data during degreening revealed that the bleached population had low survivorship and high age-specific mortality. The results emphasized the importance of secondary carotenoids for survival under nitrogen starvation.

RÉSUMÉ - Le déverdissement de *Chlorella fusca* sous des conditions de déficit en azote entraine de nombreuses modifications biochimiques telles que la perte des pigments photosynthétiques, la suppression de l'émission de l'oxygène et de la synthèse des caroténoïdes secondaires. L'addition de phenylpyridazinone herbicide BASF 44521 [4-chloro-5-methoxy-2-(α, α, α- trifluoro-m-tolyl)3(2H)-pyridazinone] à ce milieu de déverdissement inhibe la biosynthèse des caroténoïdes secondaires aboutissant à une décoloration totale et irréversible des cellules. Les analyses des données de survie durant le déverdissement ont révélé que les populations décolorées avaient un faible taux de survie et un fort taux de mortalité étroitement lié à l'âge des cellules. Ces résultats accentuent l'importance des caroténoïdes secondaires pour la survie dans des conditions de déficit en azote (traduit par la rédaction).

KEY WORDS : *Chlorella*, nitrogen starvation, secondary carotenoids, survival.

INTRODUCTION

Nitrogen starvation results in degreening of *Chlorella fusca* (Grimme & Porra, 1974). The resulting nitrogen-starved cells are photosynthetically inactive, possess non-appressed lamellar system, and can regreen and develop functional photosynthetic apparatus upon being transferred to a nitrate-rich medium (Grimme & Porra, 1974; Pyliotis *et al.*, 1975). The orange appearance of these

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cells was attributed to synthesis of the secondary carotenoids echinenone and canthaxanthin (Goodwin, 1980).

Phenylpyridazinone herbicides cause bleaching in plants (Sandmann *et al.*, 1981) by inhibiting carotenoid biosynthesis at the enzyme phytoene desaturase (Clarke *et al.*, 1982). We report here that the phenylpyridazinone herbicide BASF 44521 affects the biosynthesis of secondary carotenoids during degreening of *C. fusca*. Analysis of survivorship was carried out to evaluate the importance of secondary carotenoids for survival of *C. fusca* under conditions of nitrogen starvation.

MATERIALS AND METHODS

Chlorella fusca 211-15 from the Collection of Algal Cultures (Gottingen, Germany) was degreened in a nitrate-sparse medium (Grimme & Porra, 1974). The pure unformulated herbicide BASF 44521 [4-chloro-5-methoxy-2-(α, α, α-trifluoro-m-tolyl)-3-(2H)-pyridazinone] dissolved in acetone was added to the degreening medium to give concentrations in the range of 0.1-100 $\mu g\ ml^{-1}$ (the acetone concentration in both control and treated cultures was kept below 0.05%). The degreening process was allowed to proceed for six weeks under continuous illumination at 25°C and 200 $\mu mol\ m^{-2}\ s^{-1}$. For regreening, nitrogen-starved cells of each culture were harvested, washed, and separately resuspended in a nitrate-rich medium (Grimme & Porra, 1974) containing the same concentration of herbicide. Regreening was allowed for 32 h under conditions similar to those used for degreening.

Cell numbers were determined using a Bright-line haemocytometer (Richert-Jung, USA). Photosynthetic oxygen evolution was measured using an oxygen electrode (Rank Brothers, UK) with 5.0 ml of culture at 25°C in the electrode chamber and 800 $\mu mol\ m^{-2}\ s^{-1}$. Total chlorophyll and carotenoid contents were determined (Metzner *et al.*, 1965), secondary carotenoids were separated at the end of degreening (Chapman, 1988) and absorption spectra were recorded by using a Lumbda-2 UV/VIS spectrophotometer (Perkin-Elmer, USA). Survisorship was assessed during degreening (Pielou, 1977; Hegazy, 1990).

RESULTS

Degreened control cells appeared orange, had subtantially reduced chlorophyll and carotenoid contents, Chl a: Chl b ratio, and virtually no oxygen evolving capability (Fig. 1a-e). Absorption spectroscopy revealed the presence of new peaks at 456 and 467 nm (Fig. 2). Treatment with BASF 44521 up to a concentration of 10 $\mu g\ ml^{-1}$ did not affect cell number (Fig. 1a), resulted in further loss of chlorophylls and carotenoids, and reduction of the rate of oxygen evolution (Fig. 1b-e). Cells treated with BASF 44521 up to concentration of 10 $\mu g\ ml^{-1}$ also had new peaks at 456 and 467 nm, whereas those treated with 100 $\mu g\ ml^{-1}$ appeared bleached with no oxygen evolving capability (Fig. 1b-e), and had no specific absorption peaks (Fig. 2).

Upon regreening control cells, cell division commenced after 24 h leading to a 23-fold increase in cell number (Fig. 1f). At the end of regreening, control cells had chlorophyll and carotenoid contents, Chl a: Chl b ratio, and a rate of oxygen evolution comparable to those of normal green cells. At a concentration of 10 $\mu g\ ml^{-1}$ BASF 44521 slightly affected cell division, the regreening-induced rise of chlorophyll and carotenoid contents, Chl a: Chl b ratio, and the

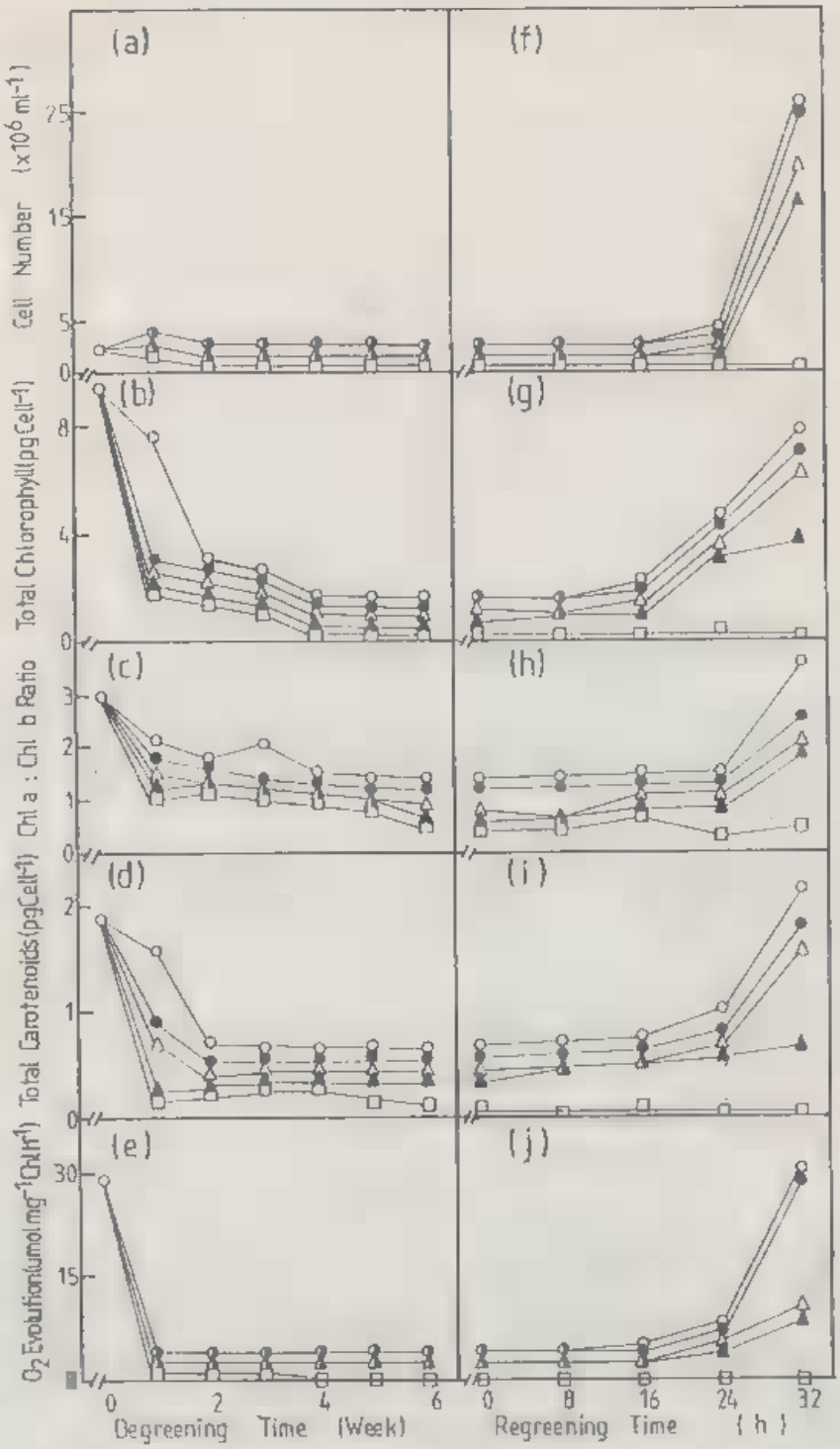

Fig. 1. - Effect of BASF 44521 on growth (cell number), pigment content, and oxygen evolution during degreening (a-e) and regreening (f-j) of *C. fusca*, (o) control, (●) 0.1, (Δ) 1.0, (▲) 10, and (□) 100 μg ml^{-1} BASF 44521 (± SE, n = 3).

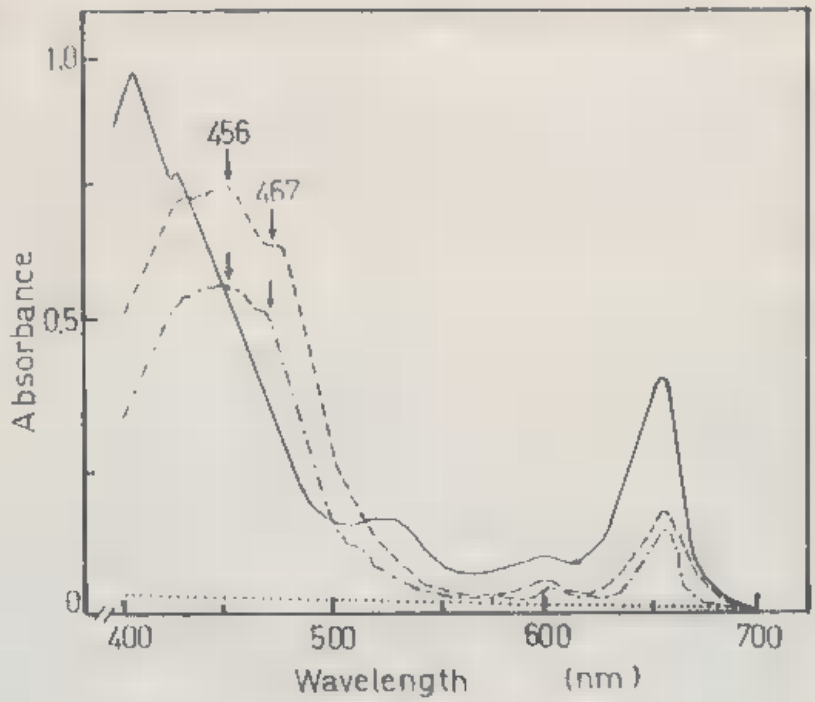

Fig. 2. - Absorption spectra of pigment extracts of green control (—), orange control (- -) *C. fusca* cells, and of cells degreened in the presence of 1.0 μg ml^{-1} (-.-) or 100 μg ml^{-1} (.....) BASF 44521. Arrows denote new absorption peaks at 456 467 nm.

restoration of oxygen evolution (Fig. 1f-j). It is important to emphasize that cells treated at 100 μg ml^{-1} BASF 44521 did not increase in number, did not regreen, and failed to restore oxygen evolution.

Survivorship revealed that herbicide-treated degreened populations had fairly stable survival (l_x) for two weeks followed by steep decline with a uniform decrease in number. The average mortality rate (q_x) indicated that treated degreened cells had high age-specific mortality (Table I).

DISCUSSION

The observed reduced chlorophyll and carotenoid contents, diminished oxygen evolution, and appearance of the secondary carotenoids echinenone and canthaxanthin indicated by the peaks at 456 and 467 nm in degreening nitrogen-starved *C. fusca* have previously been reported (Grimme & Porra, 1974; Goodwin, 1980). These control degreened cells could attain stable survival and low mortality, and could regreen and restore oxygen evolution. Addition of BASF 44521 caused acceleration of the loss of pigments during degreening. At the high concentration of 100 μg ml^{-1}, BASF 44521 caused complete bleaching and inhibition of secondary carotenoid biosynthesis. The bleached cells showed a peak at 289 nm indicative of the accumulation of phytoene (Kummel & Grimme, 1974), had declined survivorship and high mortality, and could neither regreen nor restore oxygen evolution.

Table I. - Life table of *Chlorella fusca* during degreening in the absence of BASF 44521. X = age in days, X' = first day of the following interval, X-X' = age interval (days), D_x = length of interval (days) N_x = cells surviving to X, l_x = probability of a cell at age zero will survive to day. X, q_x = average mortality rate per day.

Treatment	X-X'	D_x	N_x ($x10^4$)	l_x	q_x ($x10_{-3}$)
Control	00-07	7	230	1.00	0.62
	07-14	7	220	0.96	0.58
	14-21	7	211	0.92	0.54
	21-28	7	203	0.88	0.98
	28-35	7	201	0.87	0.07
	35-42	7	200	0.87	0.00
BASF 44521 ($100\mu gml^{-1}$)	00-07	7	115	1.00	0.86
	07-14	7	114	0.99	4.88
	14-21	7	75	0.65	8.95
	21-28	7	58	0.50	10.59
	28-35	7	15	0.13	1.91
	35-42	7	13	0.11	0.00

It is conceivable that the biochemical changes taking place during degreening are protective measures in response to the stress imposed by nutritional imbalance under nitrogen starvation. The herbicide BASF 44521 has previously been reported to inhibit carotenogenesis by inhibiting phytoene desaturation (Klarke *et al.*, 1982). Data presented here indicated that BASF 44521 inhibited the synthesis of secondary carotenoids during degreening of *C. fusca*. Inhibition of secondary carotenoid biosynthesis had serious implications for survival of *C. fusca* under nitrogen starvation. Such coloured compounds are capable of photoprotecting the photosynthetic apparatus of degreened cells. It is, therefore concluded that the synthesis of secondary carotenoids during degreening is an important aspect of a protective strategy adopted by *C. fusca* for survival under conditions of nitrogen starvation.

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